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(54) Title: CELLULAR AND SERUM PROTEIN ANCHORS FOR DIAGNOSTIC IMAGING

(57) Abstract

Methods of non-invasive diagnosis are provided employing bifunctional anchor molecules. The bifunctional anchor molecules have a functional group capable of activation which, when activated, may form a covalent bond with a reactive functionality on a target protein present in the mammalian vascular system, thereby "anchoring" the molecule to that target protein. The bifunctional anchors are also conjugated, either directly or indirectly, to a diagnostic agent of interest which provides the ability to diagnostically and non-invasively image the mammalian vascular space. Vascular targets include both cellular- and noncellular-associated proteins present in the mammalian vascular system. The methods find use for numerous applications arising from the ability to diagnostically image the mammalian vascular space over an extended period of time or to preferentially diagnostically image only a specific cell type or compartment of the mammalian vascular space.

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CELLULAR AND SERUM PROTEIN ANCHORS FOR DIAGNOSTIC IMAGING

INTRODUCTION

Technical Field

The field of this invention is the non-invasive diagnostic imaging of the mammalian vascular space.

Background

It is frequently desirable to non-invasively image the mammalian vascular space for such purposes as detecting abnormalities in blood flow, measuring cardiac function or for visualizing anatomical structures of the circulatory system. For example, in the case of certain disorders of or injuries to the vascular system which affect blood flow, one may wish to detect and visualize abnormal bleeding or, alternatively, the presence of thromboses. One may also wish to measure the effect of certain vascular disorders on cardiac efficiency and ventricular output. Additionally, non-invasive diagnostic imaging of anatomical structures of the mammalian vascular system may allow for the early detection of developmental abnormalities or various lesions, e.g., tumors, associated with the vascular system.

Present methodologies for non-invasively imaging the mammalian vascular system include such diagnostic techniques as positron emission tomography (PET), computerized tomography (CT), single photon emission computerized tomography (SPECT), magnetic

resonance imaging (MRI), nuclear magnetic imaging (NMI), fluoroscopy, ultrasound, etc. However, while these techniques are xtremely useful for a variety of different applications, they often provide far less than the desired utility, particularly when one wishes to preferentially image only a single or limited number of specific cell types or anatomical structures associated with the mammalian vascular space or to image the vascular space over an extended period of time.

There is, therefore, substantial interest in providing novel methods for enhancing the ability to preferentially image specific cell types or compartments of the mammalian vascular space and to diagnostically image the mammalian vascular space over an extended period of time.

SUMMARY OF THE INVENTION

Methods and compositions are provided for non-invasive imaging of a anatomical compartment by employing bifunctional reagents capable of covalently bonding to proteins present on the membrane of circulating blood cells or proteins in the plasma of the mammalian vascular system. The methods allow for monitoring the mammalian vascular compartment over an extended period of time. The reagent compositions of the present invention are bifunctional anchor molecules that have a functional group capable of activation which, when activated, forms a covalent bond with a reactive functionality on a target protein present in the mammalian vascular system, thereby "anchoring" the molecule to that target protein. The bifunctional anchor molecules of the present invention are also conjugated, either directly or indirectly, to a diagnostic agent of interest which provides the ability to diagnostically and non-invasively image the mammalian compartment.

The applications of the subject invention encompass MRI, CT, PET, SPECT imaging, detection of blood flow, abnormal bleeding, thromboses and vascular inflammation, vessel imaging, measuring cardiac efficiency and/or visualizing anatomical structures of the circulatory system.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods and compositions are provided for non-invasive imaging and diagnosis of anatomical compartments, particularly the vascular compartment. The methods comprise covalently bonding a diagnostic agent of interest to a protein or proteins present in the mammalian vascular system, wherein the diagnostic agent becomes bound to a long-lived protein or proteins present in the vascular system, thereby allowing one to diagnostically image the mammalian compartment space over an extended period of time and/or wherein the diagnostic agent is preferentially bound to a specific protein or limited number of proteins present in the vascular system, thereby enhancing the ability to diagnostically image only a specific compartment. The covalent bonding is achieved by administering to the vascular system of a mammalian host from one to two compounds, including at least a first compound comprising a bifunctional anchor molecule having an activated functional group capable of forming covalent bonds with reactive functionalities on a vascular protein or proteins, which is linked either to a diagnostic agent of interest or to a first binding member of a specific binding pair.

By administering the first compound to the vascular system of a mammalian host, the activated functional group will covalently bond to reactive functionalities on a protein or proteins present in the vascular system, thereby creating a population of functionalized vascular proteins. If the first compound comprises a bifunctional anchor molecule linked to a first binding member of a specific binding pair, a second compound comprising a reciprocal second binding member joined to a diagnostic agent of interest is administered to the vascular system at any time during the lifetime of the functionalized vascular proteins. After administration, the second binding member will bind to the first binding member, thereby anchoring the diagnostic agent of interest to the functionalized vascular protein or proteins. The first binding molecule can also represent a composite of both the binding compound and the diagnostic agent.

Imaging of the diagnostic agent present in the host's vascular system can then be accomplished.

Bifunctional anchor molecules comprise an active functional group capable of covalently bonding to a reactive functionality on long-lived proteins present in the mammalian vascular system, the diagnostic agent of interest or the first binding member of a specific binding pair and a linker to join the above described moieties. Long-lived vascular proteins have an *in vivo* half-life of at least about 12 hours, preferably at least about 48 hours, more preferably at least about 5 days. As such, bonding of the bifunctional anchor molecule to such proteins allows for imaging of the vascular space over an extended period of time of at least about 6 hours, preferably at least about 24 hours and more preferably at least about 3 days. Examples of long-lived proteins present in the mammalian vasculature include serum albumin, ferritin, immunoglobulin, α_1-microglobulin, α_2-macroglobulin, α-, β- or γ-globulin, thyroxine binding protein, steroid binding proteins. Specific protein targets include as part of cells include the surface membrane proteins of erythrocytes, particularly glycophorin A and C, T or B cell surface proteins, such as CD3, CD4, CD5, CD8, CD28, CD34, B7, p28, CTLA-4, Thy1, LFA1, sIgE, sIgM, IIb/IIIA, leukocyte surface membrane proteins, IL-2 receptor, integrins, serum albumin, immunoglobulins, particularly IgG and IgM, apolipoproteins, such as LDL, HDL and VLDL, endothelial cell surface proteins, including integrins, adhesion proteins, etc., or the like.

The reactive functionalities available on vascular proteins for covalent bond formation with the bifunctional anchor molecule are primarily amino, carboxyl and thiol groups. While any of these may be used as the target for the reactive functional group of the bifunctional anchor molecule, for the most part, bonds to amino groups will be employed, particularly with the formation of amide bonds.

To form amide bonds, one may employ a wide vari ty of active carboxyl groups as the reactive functional group of the bifunctional anchor molecule, particularly esters, where the hydroxyl group is physiologically

acceptable at the levels required. While a number of different hydroxyl groups may be employed, the most convenient will be N-hydroxysuccinimide and N-hydroxy sulfosuccinimide, although other alcohols, which are functional in the vascular environment may also be employed. In some cases, special reagents find use such as diazo, azido, carbodiimide anhydride, hydrazine, or thiol groups, depending on whether the reaction is *in vivo* or *in vitro*, the target, the specificity of the anchor, and the like.

The two moieties of the anchor may be joined by a bond (0 atoms in the chain) or a linker which linker is convenient, is physiologically acceptable at utilized doses and fills the requirements of the bifunctional anchor molecule, such as being stable in the vascular system, effectively presenting the diagnostic agent of interest or first binding member, allowing for ease of chemical manipulation, and the like, may be employed. The linker may be aliphatic, alicyclic, aromatic or heterocyclic, or combinations thereof, and the selection will be primarily one of convenience. The linker may be substituted with heteroatoms including nitrogen, oxygen, sulfur, phosphorus. Groups which may be employed include alkylenes, arylenes, aralkylenes, cycloalkylenes, and the like.

Generally, the linker of from 1-30, usually 1-10, more usually of from 1-6 atoms in the chain, where the chain will include carbon and any of the heteroatoms indicated above. For the most part, the linker will be straight chain or cyclic, since there normally will be no benefit from side groups. The length of the linker will vary, particularly with the nature of the diagnostic agent of interest or the first binding member, since in some instances, the diagnostic agent of interest or the first binding member may have a chain or functionality associated with it. The length of the linker may be used to provide for flexibility, rigidity, polyfunctionality, orientation, or other characteristics for improved function of the bifunctional anchor molecule. The linker may also provide for preferential bonding to a given protein epitope or sequence present in the vasculature

as compared to other proteins epitopes or sequences present in the vasculature.

A large number of small synthetic bifunctional organic compounds comprising an appropriate activatable or activated functional group are available for joining the activated functional group to the diagnostic agent of interest or to the first binding member of a specific binding pair. Illustrative compounds include: azidobenzoyl hydrazine, N-[4-(p-azidosalicylamino)butyl]-3'-[2'-pyridyldithio)propionamide, bis-sulfosuccinimidylsuberate, dimethyl adipimidate, disuccinimidyl tartrate, N-γ-maleimidobutyryloxysuccinimide ester, N-hydroxy sulfosuccinimidyl-4-azidobenzoate, N-succinimidyl [4-azidophynyl]-1,3'-dithiopropionate, N-succinimidyl [4-iodoacetyl]aminobenzoate, glutaraldehyde, succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate.

The linker joining the activated functional group and the diagnostic agent of interest or the first binding member may be oligomeric in nature and possess a high non-covalent affinity for a specific protein present in the mammalian vasculature as compared to other proteins present in the mammalian vasculature. Such oligomeric anchor molecules allow one to direct diagnostic agents of interest to specific targets, cells and/or proteins, in the vasculature, thereby allowing one to preferentially enhance the diagnostic signal in a particular anatomical compartment.

Preferably, oligomeric anchor molecules which find use will have the capability of preferentially bonding to reactive functionalities on a specific protein present in the vascular system. Preferential bonding means that the anchor molecule exhibits some preferential bonding to the vascular protein of interest as against other proteins present in the vascular environment. The preference for bonding the specified protein target will normally be at least about 1.5, preferably at least about 2 times, and may be 5 times or more as compared to random bonding in the absence of the oligomer.

The oligomeric linker of the bifunctional anchor molecule may be an oligopeptide, oligonucleotide, combinations thereof, or the like.

Generally, the number of monomeric units in an oligomeric linker will be from 4 to 12, more usually from 4 to 8 and preferably from 5 to 8. The monomer units may be naturally occurring or synth tic, generally being from about 2 to 30 carbon atoms, usually from about 2 to 18 carbon atoms and preferably from about 2 to 12 carbon atoms.

If the linker is an oligopeptide, the amino acid monomers may be naturally occurring or synthetic. Conveniently, the L-α-amino acids will be used, although the D-enantiomers may also be employed.

The amino acids employed will preferentially be free of reactive functionalities, particularly reactive functionalities which would react with the activated functional group or diagnostic agent of interest attached to the oligomeric linker. Therefore, the amino acids which are used will usually be free of reactive amino, guanidino and carboxy groups, frequently being free of hydroxy and thiol groups. Of particular interest are the naturally occurring amino acids having hydrocarbon side chains including alanine (A), glycine (G), proline (P), valine (V), phenylalanine (F), isoleucine (I) and leucine (L) or uncharged polar amino acids like methionine (M).

The amino acid monomers of an oligopeptide linker may also be synthetic. Thus, any unnatural or substituted amino acids of from 4 to 30, usually from 4 to 20, carbon atoms may be employed. Of particular interest are the synthetic amino acids β-alanine and γ-aminobutyrate or functional group protected amino acids such as O-methyl-substituted threonine (T), serine (S), tyrosine (Y), or the like.

Amino acids which find use may have the carboxyl group at a site other than the terminal carbon atom, may have the amino group at a site other than the α-position or may be substituted with groups other than oxy, thio, carboxy, amino or guanidino.

Synthetic amino acids may also be monosubstituted on nitrogen. N-substituted amino acids which find use will have an N-substituent of from about 1 to 8, usually 1 to 6 carbon atoms, which may be aliphatic, alicyclic, aromatic or heterocyclic, usually having not more

than about 3 heteroatoms, which may include amino, either tertiary or quaternary, oxy, thio, and the like.

Oligopeptide linkers are usually constructed by employing standard Merrifield solid phase synthetic techniques using an automated peptide synthesizer, standard protection chemistry (e.g., t-boc or f-moc chemistry) and resins (e.g., 4-methyl benzhydryl amine). Other synthetic techniques, however, such as liquid phase oligopeptide synthesis may also find use.

If the oligomeric linker is an oligonucleotide, either naturally occurring or synthetic nucleotide monomers may be employed. Particularly, for synthetic nucleotides, the phosphate or sugar groups may be modified where phosphate may be substituted by having the oxygen atoms replaced with sulfur or nitrogen, the phosphate group may be replaced with sulfonate, amide etc., the ribose or deoxyribose may be replaced with 5 to 6 carbon atom sugars such as arabinose, fructose, glucose, or the like, and the purines and pyrimidines may be modified by substitution on nitrogen, with alkyl or acyl, may employ different ring structures, may have nitrogen replaced by oxygen, or vice versa, and the like.

Once synthesized, an available functional group on the oligomeric linker may be activated so as to be able to covalently bond to a reactive functionality present on a vascular protein in the environment in which the reaction is to occur. The activated functional group may be present at any position on the oligomeric linker, but will usually be proximal to one or the other terminus. Conveniently, a member of the oligomer may carry the activated functional group, such as on an aspartate or glutamate moiety.

For activation of a carboxyl group on the oligomeric linker, one may use a wide variety of anhydride or ester leaving groups, where the leaving group may have oxygen or sulfur bonded to carbonyl. In instanc s where one is interested in using the oligomeric anchor molecule *in vivo*, one may select the leaving group to be physiologically acceptable.

Compounds which may be used to activate the carboxyl functional group include carbodiimides, phenols, thiophenols, benzyl alcohols, N-hydroxy imides, etc.

If the oligomeric linker is synthesized on a solid support, a functional group on the oligomer may be activated and the activated oligomer subsequently liberated from the solid support. Alternatively, the oligomer may be liberated from the support, thereby providing an available functional group for activation, and the functional group subsequently activated.

Bifunctional anchor molecules, whether comprising an oligomeric or non-oligomeric linker, are also coupled, either directly or indirectly, to a diagnostic agent of interest which imparts the ability to diagnostically image the mammalian vascular space. Preferably, the diagnostic agent is such that it does not react with the activated functional group of the bifunctional anchor molecule nor is it affected when the functional group of the anchor molecule is activated. Thus, diagnostic agents which find use generally do not react with reactive carboxyl and amino groups.

Diagnostic agents may be attached directly to the linker of the bifunctional anchor molecule, where it may be attached at any convenient site, or attached either directly or indirectly to the second binding member of a specific binding pair. Direct attachments are via a chemical bond. However, if the diagnostic agent is indirectly attached to the second binding member, it may be attached through a bond or an appropriate linking group. Depending upon the nature of the diagnostic agent employed, the linking group between the second binding member and the diagnostic agent may provide for displacement in solution of the diagnostic agent from the second binding member by having a relatively long hydrophilic linking group having heteroatoms in the chain or as side groups, .g. oxy, amino, oxo, carboxylate, etc. The linking group may be an amino acid or oligopeptide of from 2 to 6 amino acids, polyoxyalkylene of from 1 to 10 units, where alkylene is of from 2 to 3 carbon atoms, a

sugar or the like. The particular linking group employed will usually depend on the nature of the diagnostic agent and its function.

Diagnostic agents which find use include those that commonly serve as probes for known diagnostic imaging techniques such as PET, SPECT, CT, MRI, NMI, fluoroscopy, angiography, or the like. Diagnostic agents of interest include contrast agents, radioisotopes of such elements as iodine (I), including ^{123}I, ^{125}I, ^{131}I, etc., barium (Ba), gadolinium (Gd), technetium (Tc), including ^{99}Tc, phosphorus (P), including ^{31}P, iron (Fe), manganese (Mn), thallium (Tl), chromium (Cr), including ^{51}Cr, carbon (C), including ^{11}C, or the like, fluorescently labeled compounds, etc.

Generally, it will be satisfactory to have the diagnostic agent of interest bonded directly to the bifunctional anchor molecule, thereby doing away with the need to administer a second compound comprising a second binding member attached to the diagnostic agent of interest. However, in certain situations it may be preferable to attach the diagnostic agent of interest to the bifunctional anchor molecule (which is covalently bonded to a vascular protein or proteins) indirectly, i.e., through the association between a first binding member of a specific binding pair with its reciprocal second binding member. Such situations include where it is useful to covalently bond the bifunctional anchor to vascular proteins *in vivo* and then periodically administer small amounts of the second binding member/diagnostic agent complex which is cleared from the vascular system, e.g., for use of diagnostic agents which are toxic when maintained at continuously high concentrations *in vivo*.

When the diagnostic agent of interest is bonded to the bifunctional anchor molecule indirectly, the first binding member which is joined to the bifunctional anchor molecule will generally be a small molecule, where the molecule is likely to minimize any immune response. Thus, for th most part, th first binding member will be haptenic, usually b low about 1 kD and generally more than about 100 D, preferably less than about 600 D. Any physiologically acceptable molecule may be employed, where there is a convenient reciprocal second binding member.

Thus, of particular interest is biotin, where avidin or streptavidin may be the reciprocal second binding member, but other molecules such as m tal chelates, molecules mimicking a natural epitope or receptor or antibody binding site, also may find use, where the reciprocal second binding member may be an antibody or a fragment thereof, particularly a Fab fragment, an enzyme, a naturally occurring receptor, or the like. Thus, the first binding member may be a ligand for a naturally occurring receptor, a substrate for an enzyme, or a hapten with a reciprocal receptor.

The first binding member will naturally be found at low concentration, if at all, in the host vascular system, so there will be little if any competition between the first binding member and naturally occurring compounds in the vascular system for binding to the reciprocal second binding member. The reciprocal second binding member is such that it should not bind to compounds which it may encounter in the vascular system of the host.

The reciprocal second binding member of the specific binding pair will be determined by the nature of the first binding member employed. As already indicated, the second binding member may take numerous forms, particularly as binding proteins, such as immunoglobulins or fragments thereof, particularly Fab, Fv, or the like, particularly monovalent fragments, naturally occurring receptors, such as surface membrane proteins, enzymes, other binding proteins, such as avidin or streptavidin, or the like. Generally, the affinity of the second binding member for its reciprocal first binding member will be at least about 10^{-6}, more usually about 10^{-8}, e.g., binding affinities normally observed for the binding of monoclonal antibodies to their specific binding entities. Of particular interest is avidin and streptavidin, although other receptors of particular interest include receptors for steroids, LH, TSH, FSH, or their agonists, as well as sialic acid and viral hemagglutinins, and superantigens. Th second binding member will usually be a macromolecule, generally of at least about 5 kD, more usually of at least about 10 kD and usually l ss

than about 160 kD, preferably less than about 80 kD, which may be mono- or divalent in binding sites, usually monoval nt.

The vascular protein or proteins chosen as targets for reaction with the bifunctional anchor molecule will depend upon the indication desired. Thus, depending upon the vascular target or targets chosen, the diagnostic agent can be bonded to long-lived proteins and dispersed substantially throughout the entire vasculature, in which case the indication of choice will be diagnostic imaging of the vascular system over an extended period of time, or preferentially localized to specific areas of the vascular system, in which case the indication of choice will be the preferential diagnostic imaging of a specific anatomic compartment, either with or without imaging over an extended period of time. The target may be fixed or mobile; that is substantially fixed in position, as in the case of endothelial cells, or mobile in the vascular system, i.e., not having a fixed situs for an extended period of time, generally not exceeding 5, more usually, one minute. Target cells and proteins may have a substantially uniform or variant distribution in the vascular system, where the target may preferentially localize or be concentrated in particular compartments, as compared to the vascular system or other anatomic compartments.

The diagnostic agent employed and the vascular protein or proteins targeted will depend upon whether one wishes to diagnostically image the anatomic compartment over an extended period of time, whether one wishes to preferentially image only a specific cell type or compartment, or both. Applications for covalently bonding a diagnostic agent of interest to a long-lived vascular protein for diagnostic imaging of the vascular space over an extended period of time are numerous and include enhancing the ability to detect abnormalities in blood flow throughout the entire mammalian vascular system, including the detection of int rnal injury causing abnormal bleeding or, alternatively, the pr senc of thromboses. For example, one may wish to image the vascular spac over an extended period of time to detect the effects of a particular

treatment while they occur, i.e., detecting the disappearance of an embolism, the stoppage of internal bleeding, or the like.

Diagnostically imaging the vascular space over an extended period of time also allows for the detection of various diseases associated with the vascular system, i.e., such as arterial blockage in the heart. Thus, diagnostically imaging the vascular system over an extended period of time may be employed to non-invasively detect a consistently reduced blood flow to the heart. Such a method also provides a means for quantitatively measuring cardiac efficiency and ventricular output volume over an extended period of time, i.e., during extended periods of exercise, or the like.

Other applications for such a method arise from the ability to non-invasively visualize anatomical structures of the mammalian vascular system and the effects on those anatomical structures over time of the administration of various drugs, such as vasodilators, vasoconstrictors, or the like. Such may allow for the early detection of developmental vascular abnormalities, injuries, or the like.

Additional applications arising from the ability to diagnostically image the vascular space over an extended period of time include functional assessment of the cardiovascular system as routinely utilized in nuclear medicine for single measurements.

The first compound and, if required, the second compound, will usually be administered as a bolus, but may be introduced slowly over time by transfusion using metered flow, or the like. Alternatively, although less preferable, blood may be removed from the host, treated *ex vivo*, and returned to the host. The first and second compounds will be administered in a physiologically acceptable medium, e.g., deionized water, phosphate buffered saline, saline, mannitol, aqueous glucose, alcohol, vegetable oil, or the like. Usually, a single injection will be employed although more than one injection may be used, if desired. The first and second compounds may be administered by any convenient means, including syringe, catheter, or the like. The particular manner of

administration will vary depending upon the amount to be administered, whether a single bolus, sequential, or continuous administration, or the like. Administration will be intravascular, where the sit of introduction is not critical to this invention, preferably at a site where there is rapid blood flow, e.g., intravenously, peripheral or central vein. The intent is that the compound administered be effectively distributed in the vascular system so as to be able to react with target proteins therein.

The dosage of the compound will depend upon whether it comprises the diagnostic agent of interest and will, therefore, be dependent on the adverse effects of the diagnostic agent of interest, if any, the time necessary to reduce the unbound concentration of the agent present in the vascular system, the dosage necessary for successful diagnostic imaging, the indication being sought, the sensitivity of the diagnostic agent to destruction by vascular components, the route of administration, and the like. As necessary, the dosage of diagnostic agent may be determined empirically, initially using a small multiple of the dosage normally administered, and as greater experience is obtained, enhancing the dosage. Dosages will generally be in the range of 1 ng/Kg to 10 mg/Kg, usually being determined empirically in accordance with known ways, as provided for in preclinical and clinical studies.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for non-invasively imaging an anatomical compartment of a mammalian host, said method comprising:

administering to the vascular system of said mammalian host a first entity comprising a bifunctional anchor molecule characterized by having (i) a reactive functional group which reacts with long-lived vascular proteins to form covalent bonds joined to (ii) an agent, wherein said reactive functional group reacts with at least one of long-lived protein and cellular components of said vascular system to produce modified vascular components, wherein said agent is a diagnostic imaging agent or a member of a specific binding pair, and when said agent comprises said member of said specific binding pair, said method comprises the further step of adding a second entity, comprising a diagnostic imaging agent and the reciprocal member of said specific binding pair, whereby said compound is retained in said anatomical compartment for an extended period of time or concentrated in said anatomical compartment; and

imaging said anatomical compartment by means of said diagnostic imaging agent.

2. A method according to Claim 1, wherein said reactive functional group is a carboxylate ester which reacts with amines in aqueous medium to form amides.

3. A method according to Claim 1, wherein said reactive functional group of said bifunctional anchor molecule covalently bonds to a long-lived protein on the surface of at least one of erythrocytes and platelets.

4. A method according to Claim 1, wherein said r activ functional group of said bifunctional anchor molecule covalently bonds to a

long-lived protein selected from the group consisting of serum albumin, transferrin and immunoglobulin.

5. A method according to Claim 1, wherein said diagnostic agent of interest is a radioactive isotope.

6. A method according to Claim 5, wherein said radioactive isotope is of an element selected from the group consisting of iodine, technetium, gadolinium, chromium or barium.

7. A method for non-invasively imaging the vascular space of a mammalian host over an extended period of time, said method comprising:

contacting erythrocytes and/or platelets of the mammalian host *ex vivo* with a compound comprising a bifunctional anchor molecule characterized by having (i) a reactive functional group which reacts with proteins to form covalent bonds joined to (ii) a diagnostic agent of interest, wherein said reactive functional group reacts with at least one long-lived protein on the surface of said erythrocytes and/or platelets to produce modified erythrocytes and/or platelets;

introducing said modified erythrocytes and/or platelets into the vascular system of said mammalian host; and

imaging said diagnostic compound of interest present in said vascular system.

8. A method for non-invasively imaging the vascular space of a mammalian host over an extended period of time, said method comprising:

administering to the vascular system of a mammalian host a first compound comprising a bifunctional anchor molecule characterized by having (i) a reactive functional group which reacts with long-lived vascular proteins to form covalent bonds joined to (ii) a first binding member which

is a member of a specific binding pair consisting of said first binding member and a reciprocal second binding member, wherein said reactiv functional group reacts with at least one of long-lived protein and cellular components of the vascular system to produce modified vascular components;

administering to the vascular system of said mammalian host a second compound comprising (i) said reciprocal second binding member joined to (ii) a diagnostic agent of interest, wherein said reciprocal second binding member binds to said first binding member of said modified vascular components; and

imaging said diagnostic agent of interest present in said vascular system.

9. A method according to Claim 8, wherein said reactive functional group is a carboxylate ester which reacts with amines in aqueous medium to form amides.

10. A method according to Claim 8, wherein said first binding member is biotin and said second binding member is selected from the group consisting of avidin or streptavidin.

11. A method according to Claim 8, wherein said reactive functional group of said bifunctional anchor molecule covalently bonds to a long-lived protein on the surface of cells, wherein said cells are selected from the group consisting of erythrocytes and platelets.

12. A method according to Claim 8, wherein said reactive functional group of said bifunctional anchor molecule covalently bonds to a long-lived protein selected from the group consisting of serum albumin, transferrin and immunoglobulin.

13. A method according to Claim 8, wherein said diagnostic agent of interest is a radioactive isotope.

14. A method according to Claim 13, wherein said radioactive isotope is of an element selected from the group consisting of iodine, technetium, gadolinium, chromium or barium.